

Electronic supplementary information

**Ultrasensitive melanoma biomarker detection using a microchip SERS
immunoassay with anisotropic Au-Ag alloy nanoboxes**

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Table S1: Comparison of the performance of commercial ELISA kit and a microchip SERS immunoassay method.

Item	Commercial ELISA method	Our work
Linear Range	156-10000 pg/ml	200-800 pg/ml
Limit of detection	94 pg/ml	50 pg/ml
Time Required	7 hrs	5 hrs
Steps Involved	14 steps	6 steps
Sample Volume Requirement	100 μ l	50 μ l

Table S2: Comparison of microchip SERS immunoassay sensitivity to other methods for detecting melanoma biomarkers.

Melanoma-associated Biomarker	Assay Type	Detection Method	Sensitivity	Reference numbers in the manuscript
CEACAM-1 (Carcinoembryonic antigen cell adhesion molecule - 1)	Magnetic Immunoassay (MIA)	Fluorescence Spectroscopy	351.2 pg/ml	26
OPN (Osteopontin)	Magnetic Immunoassay	Fluorescence	278.8 pg/ml	26
LDH (Lactate dehydrogenase)	Protein standard absolute quantification	Mass Spectroscopy	510 ng/ml	27
VEGF (Vascular Endothelial growth factor)	Fluorescence Immunoassay	Fluorescence Spectroscopy	2.5ng/ml	28

Fig. S1: Western blot images showing the presence of MCSP in MEL 28 cells.

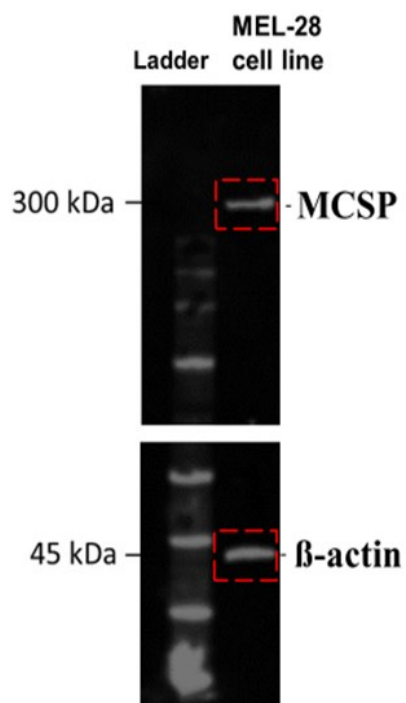


Fig. S2: Design and dimensions of the asymmetric electrodes for the multiplex microchip biosensor.

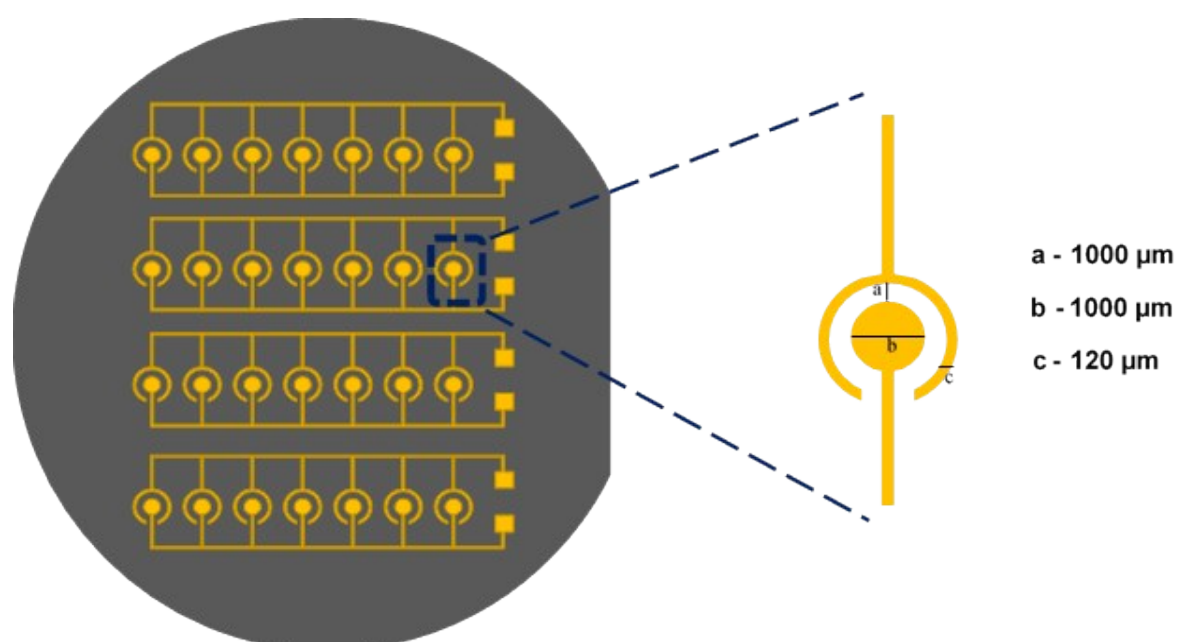
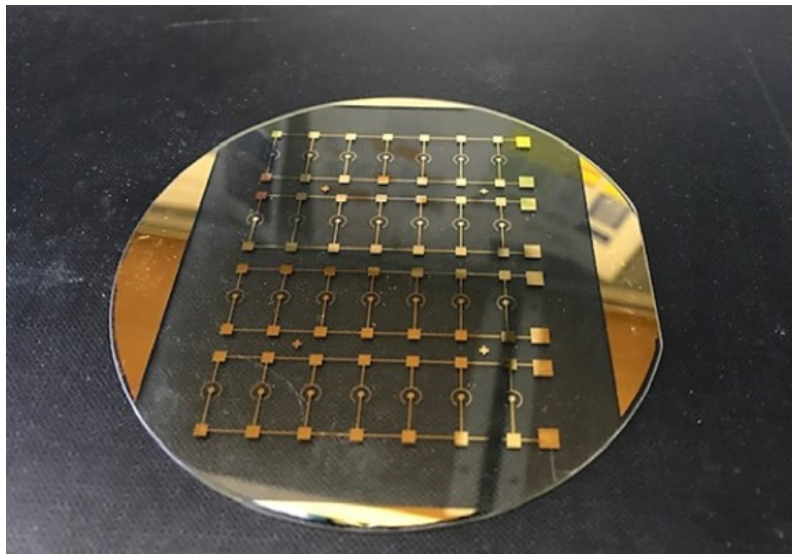
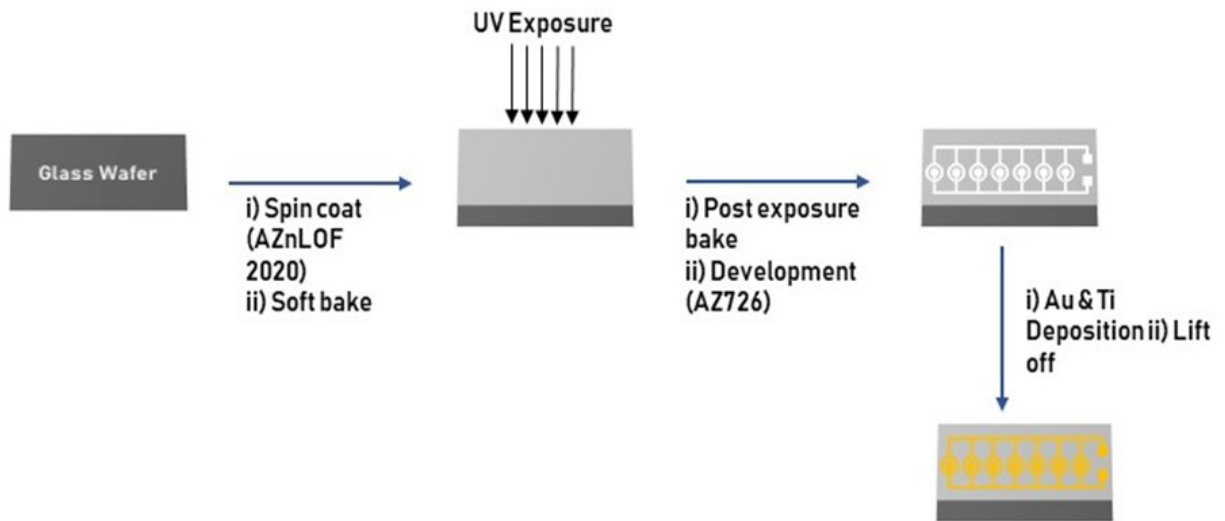


Fig. S3: Schematic of Biosensor fabrication process



Multiplex Microchip Biosensor

Fig. S4: SEM and TEM images of Au-Ag alloy Nanoboxes

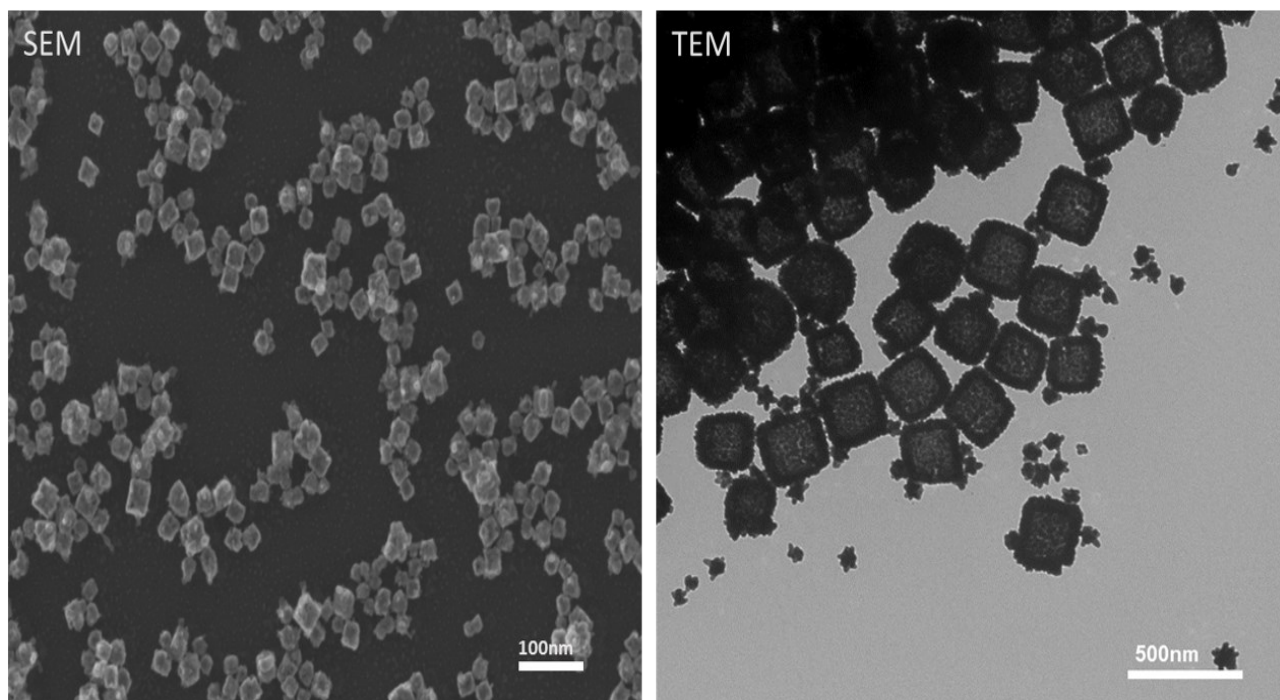


Fig. S5: Raman Spectrum of MBA

Entire Raman Spectrum

